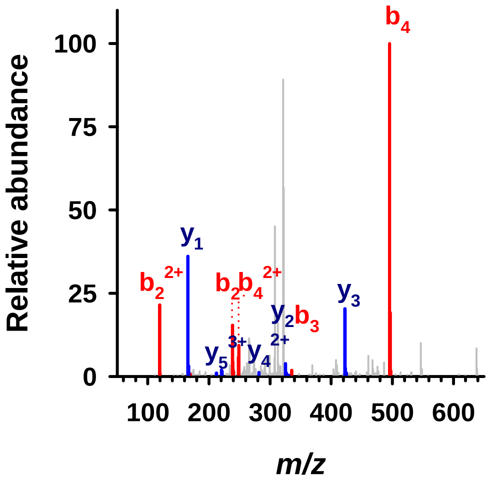
Supplemental Figures

Supplemental Figure S1. Tandem mass spectra of cysteine-containing peptides identified from the trypsin/chymotrypsin-digested CueP proteins. The corresponding peaks of the precursor MS2 ions with the given average mass (*m/z*) and charge state (z) in parenthesis are shown by extracted ion chromatograms in the main Fig. 2A with the following assignments: **a,** $^{93}\mathrm{T\underline{H}P\underline{C}F}^{97}$; **b,** $^{98}\mathrm{N\underline{H}SLSG\underline{C}GGEMPNKPF}^{113}$; and **c,** $^{158}\mathrm{TASHAIATSDDSQT\underline{C}LTELPLR}^{179}$. The subscripts following symbols of the peaks represent modifications at the underlined cysteine (C) and histidine (H) residues: IAA or NEM, alkylation of cysteine with iodoacetamide or *N*-ethylmaleimide; H, protonation of histidine at position 94 (a_H) or 99 (b_H); and -SS-, disulfide bond. Assigned *b*-ions and *y*-ions generated by collision-induced fragmentation of the precursor. Ions are shown in the following tandem mass spectra and tables with the one-letter amino acid codes.

(a) Tandem mass spectra of the modified peptides of ^{93}THPCF^{97} in the panels a and d of extracted ion chromatograms.

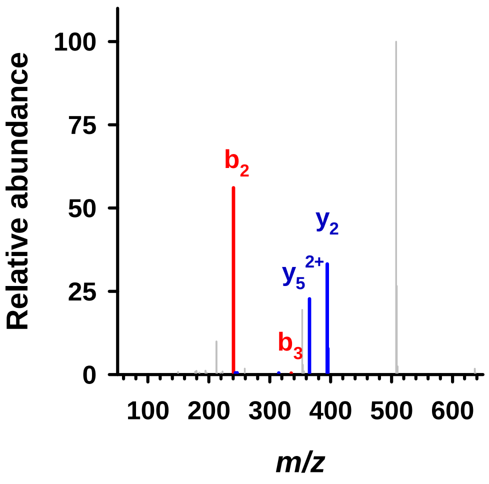
a_{IAA}: carbamidomethyl cysteine C^{CAM} at Cys96 (m/z = 331.36, z =+2)



#	b	b++	Seq.	y	y++	y+++	#
1			T	661.72	**331.36**	**221.24**	5
2	**239.25**	**120.13**	H	**560.62**	**280.81**		4
3	**336.37**	**168.69**	P	**423.48**	**212.24**		3
4	**496.53**	**248.77**	C^{CAM}	**326.36**	163.68		2
5			F	**166.2**			1

SEQUEST result:
Matches = 31, S_pScore = 381.3

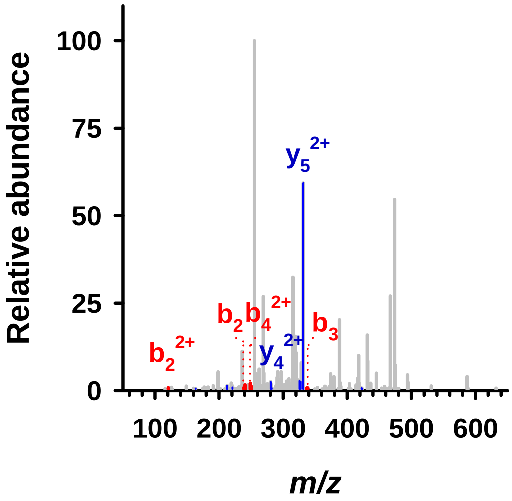
a_{NEM}: N-ethylmaleimide cysteine C^{NEM} at Cys96 (m/z = 365.42, z =+2)



#	b	b++	Seq.	y	y++	y+++	#
1			T	729.83	**365.42**	**243.95**	5
2	**239.25**	120.13	H	628.72	**314.87**	210.25	4
3	**336.37**	**168.69**	P	491.58	**246.3**		3
4	**564.64**	282.82	C^{NEM}	**394.47**	**197.74**		2
5			F	**166.2**			1

SEQUEST result:
Matches = 25, S_pScore = 206.3

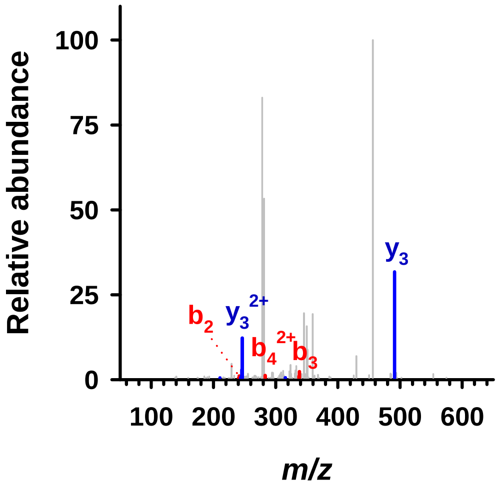
$a_{H,IAA}$: protonated histidine H^H at His94 and carbamidomethyl cysteine C^{CAM} at Cys96 (*m/z* = 331.87, *z* = +2)



#	b	b++	Seq.	y	y++	y+++	#
1			T	662.73	331.87	221.58	5
2	240.26	120.63	H^H	561.62	281.32		4
3	337.37	169.19	P	423.48	212.24		3
4	497.54	249.27	C^{CAM}	326.36	163.68		2
5			F	166.2			1

SEQUEST result:
Matches = 31, S_pScore = 363.4

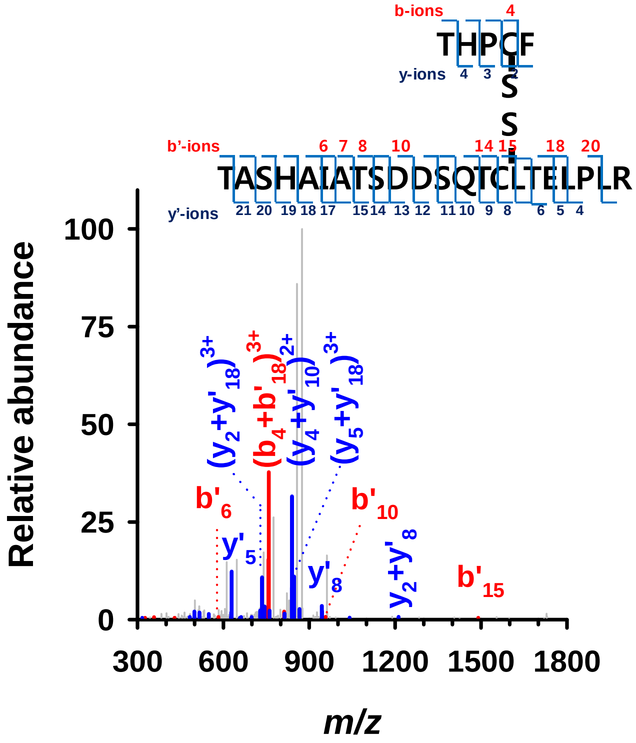
$a_{H,NEM}$: protonated histidine H^H at His94 and N-ethylmaleimide cysteine C^{NEM} at Cys96 (*m/z* = 365.92, *z* = +2)



#	b	b++	Seq.	y	y++	y+++	#
1			T	730.84	365.92	244.28	5
2	240.26	120.63	H^H	629.73	315.37	210.58	4
3	337.37	169.19	P	491.58	246.3		3
4	565.65	283.33	C^{NEM}	394.47	197.74		2
5			F	166.2			1

SEQUEST result:
Matches = 27, S_pScore = 248.7

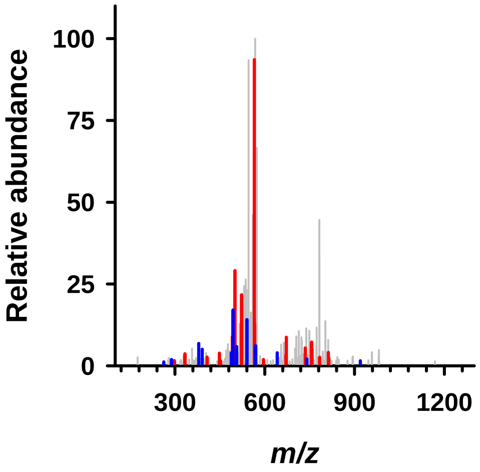
a-SS-c: disulfide bond between Cys96 and Cys172 (m/z = 978.41, z =+3)



SEQUEST result:
Matches = 63, S_pScore = 218

(b) Tandem mass spectra of the modified peptides of ^{98}NHSLSGCGGEMPNKPF^{113} in the panels b and e of extracted ion chromatograms.

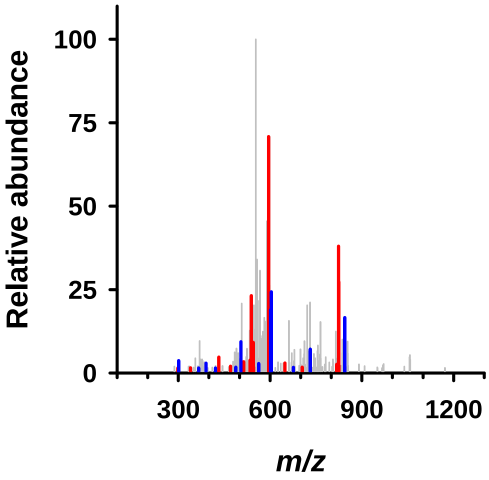
b_{IAA}: carbamidomethyl cysteine CCAM at Cys104 (m/z = 578.30, z = +3)



#	b	b++	b+++	Seq.	y	y++	y+++	#
1				N	1732.89	866.95	578.3	16
2	252.25	126.63		H	1618.78	809.9	**540.27**	15
3	339.33	170.17		S	1481.64	**741.33**	**494.55**	14
4	**452.49**	226.75		L	1394.57	697.79	465.53	13
5	**539.56**	270.28		S	1281.41	**641.21**	427.81	12
6	**596.61**	**298.81**		G	1194.33	597.67	398.78	11
7	**756.78**	**378.89**	252.93	C^{CAM}	1137.28	**569.14**	**379.77**	10
8	**813.83**	**407.42**	271.95	G	977.12	**489.06**	326.38	9
9	870.88	435.94	290.97	G	**920.06**	460.54	307.36	8
10	1000	**500.5**	**334**	E	863.01	432.01	**288.34**	7
11	1131.19	**566.1**	377.74	M	733.9	367.45	245.3	6
12	1228.31	614.66	410.11	P	602.7	301.85	201.57	5
13	1342.41	**671.71**	**448.14**	N	**505.59**	253.3		4
14	1470.58	**735.8**	**490.87**	K	**391.48**	196.25		3
15	1567.7	**784.35**	**523.24**	P	**263.31**	132.16		2
16				F	166.2			1

SEQUEST result:
Matches = 58, S_pScore = 330.6

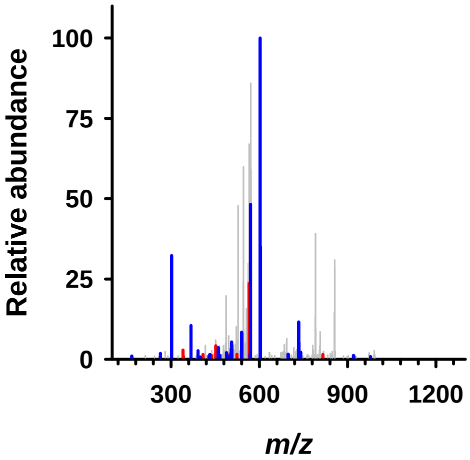
b_{NEM}: N-ethylmaleimide cysteine C^{NEM} at Cys104 (m/z = 601.00, z = +3)



#	b	b++	b+++	Seq.	y	y++	y+++	#
1				N	1801	901	**601**	16
2	252.25	126.63		H	1686.89	**843.95**	**562.97**	15
3	**339.33**	170.17		S	1549.75	775.38	517.26	14
4	452.49	226.75		L	1462.68	**731.84**	**488.23**	13
5	**539.56**	270.28		S	1349.52	**675.26**	450.51	12
6	**596.61**	**298.81**		G	1262.44	631.72	**421.48**	11
7	**824.89**	412.95	275.63	C^{NEM}	1205.39	**603.2**	402.47	10
8	881.94	441.47	294.65	G	977.12	489.06	326.38	9
9	938.99	**470**	313.67	G	920.06	460.54	307.36	8
10	1068.1	**534.56**	356.71	E	863.01	**432.01**	288.34	7
11	1199.3	**600.15**	400.44	M	733.9	**367.45**	245.3	6
12	1296.42	**648.71**	**432.81**	P	**602.7**	**301.85**	201.57	5
13	1410.52	**705.76**	**470.84**	N	**505.59**	253.3		4
14	1538.69	769.85	**513.57**	K	**391.48**	196.25		3
15	1635.81	**818.41**	**545.94**	P	263.31	132.16		2
16				F	166.2			1

SEQUEST result:
Matches = 57, S_pScore = 304.3

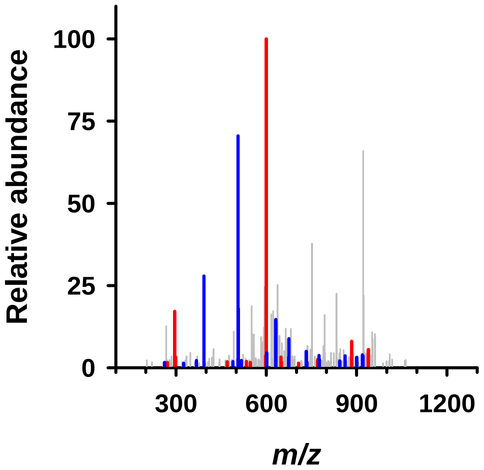
$b_{H,IAA}$: protonated histidine H^H at His99 and carbamidomethyl cysteine C^{CAM} at Cys104 (*m/z* =578.64, *z* = +3)



#	b	b++	b+++	Seq.	y	y++	y+++	#
1				N	1733.9	867.45	578.64	16
2	253.26	127.13		H^H	1619.79	810.4	**540.6**	15
3	**340.34**	170.67		S	1481.64	**741.33**	**494.55**	14
4	**453.49**	227.25		L	1394.57	**697.79**	**465.53**	13
5	**540.57**	270.79		S	1281.41	641.21	**427.81**	12
6	597.62	299.31		G	1194.33	597.67	**398.78**	11
7	757.79	379.4	253.27	C^{CAM}	1137.28	**569.14**	379.77	10
8	**814.84**	**407.92**	272.28	G	**977.12**	**489.06**	326.38	9
9	871.89	**436.45**	291.3	G	**920.06**	**460.54**	307.36	8
10	1001	**501.01**	334.34	E	863.01	**432.01**	288.34	7
11	1132.2	**566.6**	378.07	M	**733.9**	**367.45**	245.3	6
12	1229.32	615.16	410.44	P	**602.7**	**301.85**	201.57	5
13	1343.42	672.21	448.48	N	**505.59**	253.3		4
14	1471.59	**736.3**	491.2	K	**391.48**	196.25		3
15	1568.71	784.86	**523.57**	P	**263.31**	132.16		2
16				F	**166.2**			1

SEQUEST result:
Matches = 62, S_pScore = 429.7

$b_{H,NEM}$: protonated histidine H^H at His99 and N-ethylmaleimide cysteine C^{CAM} at Cys104 (*m/z* =601.34, *z* = +3)

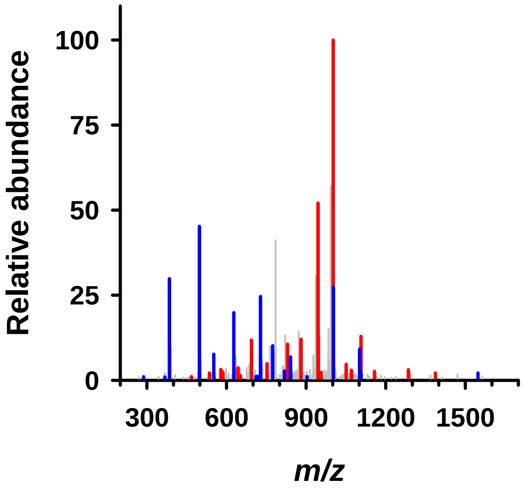


#	b	b++	b+++	Seq.	y	y++	y+++	#
1				N	1802	**901.51**	**601.34**	16
2	253.26	127.13		H^H	1687.9	**844.45**	563.3	15
3	340.34	170.67		S	1549.75	**775.38**	**517.26**	14
4	453.49	227.25		L	1462.68	**731.84**	488.23	13
5	540.57	**270.79**		S	1349.52	**675.26**	450.51	12
6	**597.62**	**299.31**		G	1262.44	**631.72**	421.48	11
7	825.9	413.45	275.97	C^{NEM}	1205.39	603.2	402.47	10
8	**882.95**	441.98	**294.99**	G	977.12	**489.06**	**326.38**	9
9	**940**	**470.5**	314	G	**920.06**	460.54	307.36	8
10	1069.11	**535.06**	357.04	E	**863.01**	432.01	288.34	7
11	1200.31	**600.66**	400.77	M	**733.9**	**367.45**	245.3	6
12	1297.42	**649.22**	433.15	P	**602.7**	301.85	201.57	5
13	1411.53	**706.27**	471.18	N	**505.59**	253.3		4
14	1539.7	**770.35**	513.9	K	**391.48**	196.25		3
15	1636.82	818.91	**546.28**	P	**263.31**	132.16		2
16				F	166.2			1

SEQUEST result:
Matches = 54, S_pScore = 333.1

(c) Tandem mass spectra of the modified peptides of ^{158}TASHAIATSDDSQTCLTELPLR^{179} in the panels c and f of extracted ion chromatograms.

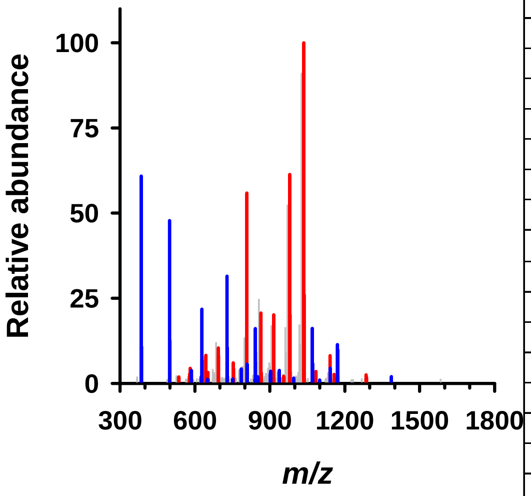
c_{IAA}: carbamidomethyl cysteine C^{CAM} at Cys172 (*m/z* = 796.86, *z* = +3)



#	b	b++	b+++	Seq.	y	y++	y+++	#
1				T	2388.56	1194.78	796.86	22
2	173.19			A	2287.45	1144.23	763.16	21
3	260.27	130.64		S	2216.38	**1108.69**	739.46	20
4	397.41	199.21		H	2129.3	1065.15	**710.44**	19
5	**468.48**	234.75		A	1992.16	996.58	664.72	18
6	**581.64**	291.32		I	1921.08	961.04	641.03	17
7	**652.72**	326.86	218.24	A	1807.92	**904.47**	603.31	16
8	**753.82**	377.42	251.95	T	1736.85	868.93	**579.62**	15
9	**840.9**	420.95	280.97	S	1635.74	**818.37**	545.92	14
10	**955.99**	478.5	319.33	D	**1548.66**	**774.84**	516.89	13
11	**1071.08**	**536.04**	357.7	D	1433.58	**717.29**	478.53	12
12	**1158.15**	**579.58**	**386.72**	S	1318.49	659.75	440.17	11
13	**1286.28**	**643.65**	429.43	Q	1231.41	616.21	411.14	10
14	**1387.39**	**694.2**	463.13	T	**1103.28**	**552.15**	**368.43**	9
15	**1547.55**	**774.28**	516.52	C^{CAM}	**1002.18**	501.59	334.73	8
16	1660.71	**830.86**	554.24	L	**842.01**	421.51	281.34	7
17	1761.81	**881.41**	**587.94**	T	**728.86**	364.93	243.62	6
18	1890.93	**945.97**	630.98	E	**627.75**	314.38	209.92	5
19	2004.09	**1002.55**	668.7	L	**498.64**	249.82		4
20	2101.2	**1051.1**	701.07	P	**385.48**	193.24		3
21	2214.36	**1107.68**	738.79	L	**288.37**	144.69		2
22				R	175.21			1

SEQUEST result:
Matches = 90, S_pScore = 961.6

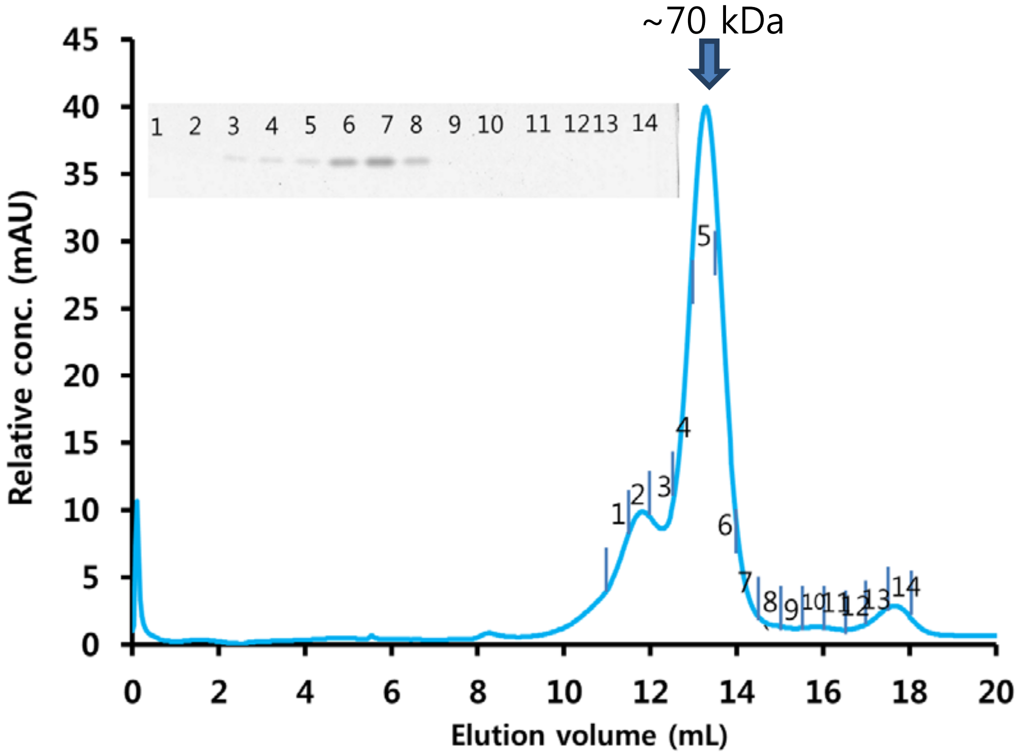
$\mathrm{c}_{\mathrm{NEM}}$: N-ethylmaleimide cysteine $\mathrm{C}^{\mathrm{NEM}}$ at Cys172 (m/z = 819.56, z = +3)



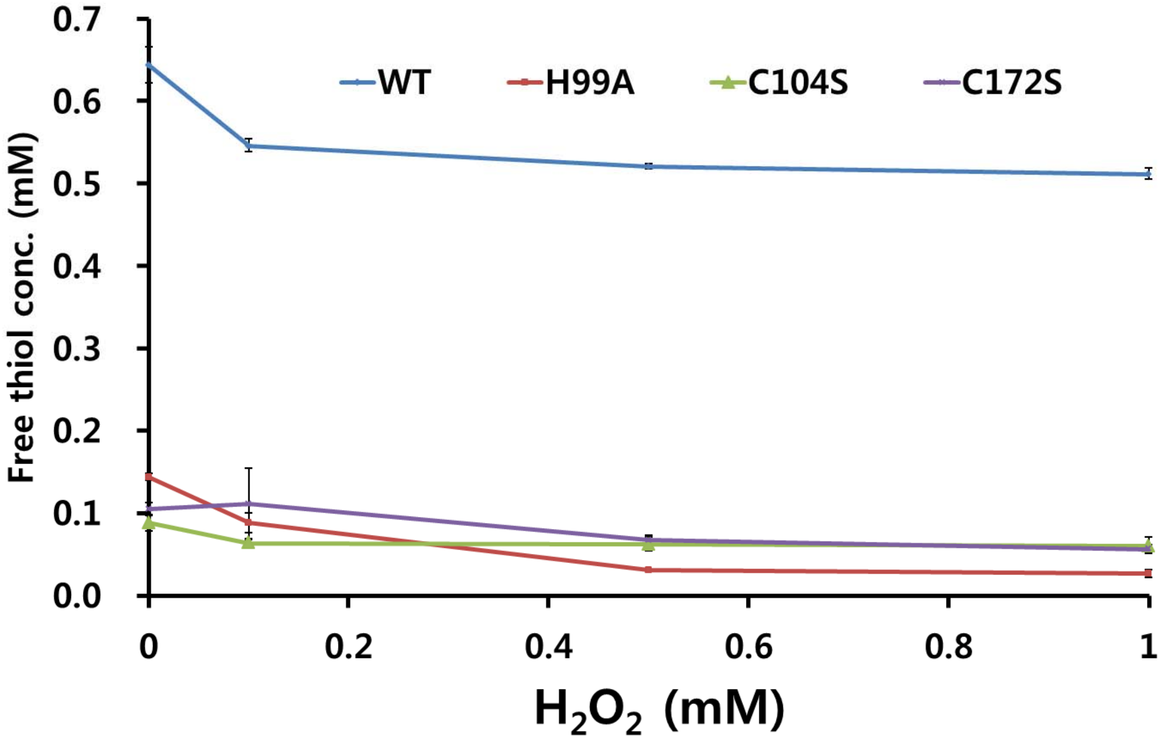
#	b	b++	b+++	Seq.	y	y++	y+++	#
1				T	2456.67	1228.84	819.56	22
2	173.19			A	2355.56	1178.29	**785.86**	21
3	260.27	130.64		S	2284.48	**1142.8**	762.17	20
4	397.41	199.21		H	2197.41	**1099.2**	733.14	19
5	468.48	234.75		A	2060.27	1030.64	687.43	18
6	**581.64**	291.32		I	1989.19	**995.1**	663.73	17
7	**652.72**	326.86	218.24	A	1876.03	**938.52**	**626.02**	16
8	**753.82**	377.42	251.95	T	1804.95	**902.98**	602.32	15
9	**840.9**	420.95	280.97	S	1703.85	**852.43**	568.62	14
10	**955.99**	478.5	319.33	D	1616.77	**808.89**	539.6	13
11	**1071.08**	**536.04**	357.7	D	1501.69	**751.35**	501.23	12
12	**1158.15**	**579.58**	**386.72**	S	**1386.6**	**693.8**	462.87	11
13	**1286.28**	**643.65**	429.43	Q	1299.52	**650.26**	433.85	10
14	**1387.39**	**694.2**	463.13	T	**1171.39**	**586.2**	391.14	9
15	1615.66	**808.33**	539.23	$\mathrm{C}^{\mathrm{CAM}}$	**1070.29**	**535.65**	357.43	8
16	1728.82	**864.91**	**576.94**	L	**842.01**	421.51	281.34	7
17	1829.92	**915.46**	610.65	T	**728.86**	364.93	243.62	6
18	1959.04	**980.02**	**653.68**	E	**627.75**	314.38	209.92	5
19	2072.19	**1036.6**	691.4	L	**498.64**	249.82		4
20	2169.31	**1085.16**	723.77	P	**385.48**	193.24		3
21	2282.47	**1141.74**	761.49	L	288.37	144.69		2
22				R	175.21			1

SEQUEST result:
Matches = 103, $\mathrm{S_p}$Score = 1335.4

Supplemental Figure S2. Elution profiles from a size exclusion chromatographic column, showing the molecular size of the CueP C172S protein. CueP variant protein (C172S) was purified in freshly prepared 20 mM Tris (pH 8.0) buffer containing 150 mM NaCl and 2 mM 2-mercaptoethanol. The protein sample was dialyzed against highly-degassed 20 mM Tris (pH 8.0) buffer containing 150 mM NaCl to remove the reducing agent. The protein sample (1 mg/ml, 100 μl) was injected on the size exclusion chromatographic column (Superdex 200 10/30) at a flow rate of 0.5 ml/min using the same degassed buffer. Each fraction was analyzed on SDS-PAGE (insets). The calculated molecular size was ~70 kDa, which is twice than the wild type protein (~34 kDa; Fig. 2C)



Supplemental Figure S3. Reactivity of CueP variants to H_2O_2. The same procedures were used as in Fig. 4B.



Supplemental Figure S4. Expression of CueP in *cusA*-deleted *E. coli* strain BW25112. The *E. coli* strain harboring the plasmid was grown exponentially, and then 5 µg of cell lysate was applied to SDS-PAGE. Expressed CueP proteins were visualized by western blot using anti-CueP antibody (*left*). As a loading control, the SDS-polyacrylamide gel stained by coomassie blue is shown (*right*). EV indicates the empty vector, and CueP indicates the cueP-containing vector. * indicates the purified CueP protein (50 ng). This figure is for Fig. 5D.